HYPERTENSIN IN THE SYSTEMIC BLOOD OF ANIMALS WITH EXPERIMENTAL RENAL HYPERTENSION

By FRANK GOLLAN, M.D., EVELYN RICHARDSON, AND HARRY GOLDBLATT, M.D.

(From the Institute of Pathology, Western Reserve University, Cleveland, and the Institute for Medical Research, Cedars of Lebanon Hospital, Los Angeles)

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The final proof for the correctness of the present view that experimental renal hypertension is caused by a humoral mechanism of renal origin would be the demonstration of the presence of a pressor substance of renal origin in the circulating systemic blood of animals with persistent hypertension of this type. The proof that essential human hypertension is of similar origin would be the demonstration of the corresponding substance in the blood of human beings with this type of hypertension.

Although there are many reasons for assuming that *in vivo* the proteolytic action of the enzyme renin on a pseudoglobulin (hypertensinogen) in the plasma results in the formation of the vasoconstrictor substance hypertensin, a polypeptide, nevertheless the final proof for the existence of this humoral mechanism in the circulating systemic blood of animals with persistent, benign, experimental renal hypertension has been lacking until now, and some investigators have asserted that in the later stages a neurogenic factor is responsible for the persistence of the elevated blood pressure. The present publication deals with the demonstration and identification of a pressor substance (hypertensin) in the systemic blood of animals in the earlier period of experimental renal hypertension.

The development of the method for the detection of hypertensin in the blood was based on the finding of Bean (1) that the enzyme hypertensinase, which destroys hypertensin, is almost without activity at 0°C. By the elimination of hypertensinase activity immediately after the withdrawal of the systemic blood, and the immediate separation and precipitation of the plasma, it should be possible to detect any hypertensin present in the circulating blood at the time the specimen of blood is withdrawn. Furthermore, it has been shown by Sapirstein and collaborators (2) that at 0°C. renin continues to act on renin substrate, although at a diminished rate, and that the reaction reaches equilibrium in about 2 hours. Thus, by prolonged incubation of the plasma, in the cold, the action of a small amount of renin on the renin substrate in the blood can be enhanced, *in vitro*, and the presence of renin detected without the addition of renin substrate.

Method

With 50 cc. syringes containing heparin solution, 200 cc. of blood was drawn rapidly from the jugular vein or femoral artery of a dog weighing 12 to 15 kilos. An 18 gauge needle was used, so that the withdrawal of the entire amount was completed in less than 5 minutes. Every syringeful of blood was immediately chilled by being emptied into a 250 cc. centrifuge cup standing in an acetone and dry ice bath, at −20°C. During the process of cooling, the blood was stirred constantly by a bent glass rod attached to an air-driven stirrer. When the entire amount of blood had been put into the centrifuge cup, no more dry ice was added to the bath, the temperature of which was usually about −10°C. when that of the blood was about 5°C. The entire 200 cc. of blood was cooled to 0°C. in not more than 10 minutes. The moment the blood had reached 0°C., the stirring was stopped and the centrifuge bottle containing the blood was removed instantly from the ice bath in order to avoid freezing of the blood. The blood was then centrifuged at 0°C. for 30 minutes at 15,000 R.P.M. in a brine-cooled centrifuge. After centrifugation the plasma usually had a temperature of about 7°C. The plasma, which was not hemolyzed, if freezing had been avoided, was drawn off by means of a previously cooled syringe, transferred to a cooled 250 cc. centrifuge bottle, and left standing in a cooler at 0°C. for 24 hours. Two hours at 0°C. was the minimum incubation time for the optimum yield of hypertensin. At the end of that time the plasma was transferred to a 250 cc. beaker previously cooled in a deep freezer at −20°C. The beaker was placed in a dish containing a dry ice and acetone mixture at −10°C. The pH of the plasma was now adjusted to 4.5 with 10 N HCl solution by use of glass electrodes. About one-fourth of the cold plasma was poured into another 250 cc. beaker previously heated in a bath of boiling water. The plasma was stirred constantly while it was being coagulated, and, when the temperature had reached 70°C., another fourth of the cold plasma was added. The heating was continued until the temperature of the plasma had reached at least 90°C. In this way the hypertensinase of the first fourth of the plasma acted at body temperature for a very short time. At pH 4.5 a clear supernatant fluid resulted after heat coagulation of the plasma proteins. This liquid was filtered off with suction through a small Büchner funnel. The pH of the clear filtrate was usually about 5.5 and was then adjusted to 7.4 with N/10 NaOH solution. The final volume, approximately one-fourth of the original amount of blood, or about 50 per cent of the plasma, was cooled to body temperature and injected intravenously into a trained unanesthetized dog. A 100 cc. syringe and an 18 gauge needle were used for the injection, which lasted approximately 30 seconds.

EXPERIMENTS

The Demonstration of Hypertensin in the Systemic Blood of Normal Dogs after the Intravenous Injection of Various Quantities of Renin.—It was demonstrated first that the intravenous injection of the solution prepared in the manner described above from a large quantity of the serum of an animal with normal blood pressure has no pressor effect. As a control of the experiments summarized in Table II, samples of 200 cc. of systemic blood of normal dogs that had not received an intravenous injection of renin were treated by the method described above and tested for the presence of renin and hypertensin. Table I shows that the plasma from this quantity of blood of normal dogs did not contain or develop a pressor substance in an amount detectable by the method used.

The next step was to demonstrate that the presence of renin in the circulating

blood can be detected. Since it is assumed that renin is the substance which initiates the humoral mechanism of experimental renal hypertension, the attempt was made to learn whether the result of the interaction of renin and renin substrate (hypertensinogen) *in vivo*, as well as *in vitro*, is hypertensin, which can be recovered from the circulating blood. For this purpose various quantities of standardized renin (3), of known potency, were injected into normal dogs, and the withdrawal of 200 cc. of venous blood was begun at the height of the rise of blood pressure, 2 to 3 minutes after the injection of the renin was completed. The blood was then treated in the manner described above for the demonstration of hypertensin. To demonstrate existent hypertensin, some of the specimens were coagulated by heat immediately after the separation of the plasma. In order to determine how much hypertensin would be formed by the amount of renin and hypertensinogen present in the sample,

TABLE I

Hypertensin in Systemic Blood of Normal Dogs

Dog. No.	Plasma from 200 cc. of blood (incubation at 0°C.).	Rise in blood pressure (direct mean femoral)
	hrs.	*mm.*
8-43	24 hrs.	0
10-49	" "	0
10-48	" "	0
10-47	" "	0
10-45	" "	0
10-48	" "	0

other specimens were allowed to stand at 0°C. for 24 hours before they were coagulated. The results obtained from the intravenous injection of 1 to 50 units of renin are shown in Table II. They confirm the finding of Houssay, Braun-Menendez, and Dexter (3 *a*) that intravenously injected renin can be detected in the systemic blood.

Table II shows that immediately after the intravenous injection of renin a non-protein, heat-stabile, vasopressor substance is rapidly formed, which can be isolated from the plasma, and which, when injected intravenously into a normal dog, produces a type of rise of blood pressure which is characteristic of hypertensin, *viz.* an immediate rise, which reaches a maximum in 1 minute or less, and is of short duration, 3 minutes or less. Thus, it has been shown that the result of the action of renin on the substrate in the plasma *in vivo* is the same as that which occurs *in vitro* (4). Table II shows that the amount of hypertensin found in the plasma during the first 5 minutes after the injection of renin increases with the amount of renin injected into the blood and that less failures of detection of hypertensin in the blood occur when larger amounts

of renin are injected. Even after the intravenous injection of only 1 unit of renin there is some formation of hypertensin, amounting to as much as half a unit in 200 cc. of blood, if the plasma is coagulated immediately after separa-

TABLE II

Dog No.	Weight	Units of renin injected intravenously	Plasma from 200 cc. of blood (kept at 0°C.)	Rise in blood pressure (direct mean femoral)
	lbs.			*mm.*
8-72	39	1	—	0
10-44	22	1	—	0
9-33	36	1	—	15
10-24	29	1	—	15
10-40	19	1	—	10
9-32	47	1	24 hrs.	0*
10-62	30	1	" "	30
9-32	47	1	" "	25
10-35	26	5	—	30
10-14	27	5	—	0*
9-32	47	5	—	30
10-14	27	5	—	0*
10-41	23	5	—	30
10-14	27	5	—	10
8-48	23	5	—	40
8-43	40	5	—	10
8-82	29	5	—	30
10-10	42	5	—	40
10-29	30	5	24 hrs.	50
9-43	19	5	" "	50
10-27	28	10	—	0*
9-95	32	10	—	30
3-31	48	10	—	0*
10-10	42	10	—	40
9-87	20	10	—	40
9-32	33	20	—	30
10-19	47	20	—	30
9-43	25	20	24 hrs.	60
10-36	31	50	" "	75

* These failures to demonstrate any pressor effect, and also the variations, are probably due to technical errors, or to destruction of the hypertensin during preparation.

tion, and about 1 unit if the plasma is kept at 0°C. for 24 hours before it is coagulated by heat.

The Demonstration of Hypertensin in the Systemic Blood of Dogs with Benign Experimental Renal Hypertension.—For this series of experiments dogs were made hypertensive by constriction of their main renal arteries (5). In some dogs this operation was performed unilaterally, but in most of the animals

both renal arteries were constricted and, after the blood pressure had risen to a hypertensive level, blood was withdrawn from the jugular vein and treated in the manner described above for the demonstration of hypertensin.

TABLE III

(*a*) Hypertensin in plasma of dogs with experimental hypertension after unilateral clamping of the main renal artery.

Dog No.	Weight	Period of hypertension	Blood pressure	Amount of blood	Incubation at 0°C.	Rist in blood pressure
	lbs.	*days*	*mm. Hg.*	*cc.*		*mm.*
10-30	20	3	175	190	—	0
10-39	30	4	180	400	24 hrs.	25
10-24	29	11	165	400	" "	30
10-39	30	20	185	400*	" "	0‡

(*b*) Hypertensin in plasma of dogs with experimental hypertension after bilateral clamping of the main renal arteries.

Dog. No.	Weight	Period of hypertension	Blood pressure	Amount of blood	Incubation at 0°C.	Rise in blood pressure
	lbs.		*mm. Hg*	*cc.*		*mm.*
10-45	23	2 days	125	300	—	15
10-39	30	3 "	190	70	—	5
10-27	26	5 "	140	225	24 hrs.	25
10-40	22	" "	140	300	" "	0
10-30	20	11 "	190	450	" "	15
10-30	20	19 "	180	330	" "	40
10-47	28	23 "	175	360	" "	30
9-22	24	25 "	200	400	" "	0
10-29	33	79 "	190	200	—	10
10-29	33	89 "	190	200	24 "	0
10-29	33	3 mos.	190	520	" "	20
8-67	38	18 "	190	200	—	15
4-61	37	3 yrs.	200	200	—	0

* The plasma filtrate, after precipitation, was concentrated to 17 cc. by pervaporation.

‡ Some of the failures to demonstrate a pressor effect, and also the variations, may have been the result of technical errors or destruction of the hypertensin during preparation.

After unilateral or bilateral constriction of the renal arteries, the pressor substance was detected in the systemic blood of some of the dogs with experimental benign renal hypertension that had existed for as long as 3 months (see Table III), especially when large amounts of blood were tested.

The amount of blood necessary for the detection of half to 1 unit of the pressor substance (about 200 cc.) is equal to about one-fifth of the whole blood volume of the animal. In control tests it was noticed that when an unusually large amount (500 cc.) of blood was drawn from single normal

animals, the plasma supernatant (after heat coagulation of the plasma) also gave a slight pressor effect, up to half a unit of hypertensin in 350 to 500 cc. blood. To eliminate this possibility of misinterpretation of the results, samples of not more than 200 cc. of blood were taken from each of several normal and hypertensive dogs and pooled. Each large sample was treated in the manner previously described for the demonstration of hypertensin and the final products were pooled and concentrated by pervaporation in front of a revolving fan, at room temperature, in a 2 inch cellophane tubing, from about 400 cc. to 50 cc. or less. Control tests with known amounts of diluted hypertensin showed that by this method of concentration about one-third of the hypertensin activity is lost.

TABLE IV

Hypertensin in Large Pooled Samples of Blood

Amount of pooled systemic blood	Incubation at 0°C.	Treated plasma concentrated to cc.	Rise in blood pressure (direct mean femoral)
	Normal dogs		
cc.			*mm.*
800	24 hrs.	25	0
800	" "	25	0
700	" "	35	0
1500	" "	50	0
	Hypertensive dogs		
700	24 hrs.	35	30
1500	" "	50	45
800	72 "	17	30

Table IV shows that in chronic benign experimental hypertension, hypertensin can be demonstrated in the circulating blood, if large amounts of systemic blood pooled from several animals are used.

The Demonstration of Hypertensin in Blood from the Renal Vein of an Ischemic Kidney.—It has been shown that by the addition of renin substrate to the renal vein blood from an ischemic kidney, and incubation, hypertensin can be demonstrated (6), although none can be detected without the addition of the hypertensinogen. The addition of the latter presumably allows the renin present in the blood to form more hypertensin. Yet it seemed important to demonstrate the formation of hypertensin from the renin and hypertensinogen present in the renal venous blood from an ischemic kidney, by the method described in this paper.

Table V shows that it was possible to demonstrate the existence of hypertensin and, by inference, renin, in the renal vein blood from a kidney the renal

artery of which was constricted. Renal vein blood from a normal kidney did not contain hypertensin.

In normal dogs, one renal artery was constricted by a clamp. After 4 days, when the blood pressure had risen 40 to 70 mm. Hg above normal, the dogs were anesthetized with ether and, by retroperitoneal approach, renal vein blood from the normal kidney as well as from the kidney with renal artery constricted was taken by separate rapid cannulation of both veins. The bloods were treated in the usual manner, immediately after withdrawal, and also after an incubation period of 24 hours at 0°C.

The Demonstration of Hypertensin in the Systemic Blood of Dogs with Experimental Renal Hypertension of the Malignant Type.—Both main renal arteries of dogs were constricted so that the malignant type of hypertension (7), with renal insufficiency and necrotizing arteriolitis, resulted.

TABLE V

Hypertensin in Blood from Renal Vein

	Amount of blood	Incubation of plasma at 0°C.	Rise in blood pressure (direct mean femoral)
	cc.		*mm.*
Normal renal vein blood.....	125*	—	0
" " " "	145*	24 hrs.	0
Ischemic renal vein blood.....	200	—	20
" " " "	160	24 hrs.	45

* The amount of normal blood was less, but there was not sufficient difference to account for the difference in content of pressor substance.

Table VI shows that in the malignant phase of experimental renal hypertension the existence of relatively large amounts of hypertensin in the systemic blood can be demonstrated. In 300 cc. of systemic blood of dog 10-34, weighing 20 pounds, 6 units of hypertensin were detected, after incubation for 24 hours.

The Effect of Hypertensinase on the Pressor Substance Derived from the Systemic Blood of a Normal Dog, after the Intravenous Injection of Renin, and from the Systemic Blood of a Dog with Malignant Hypertension.—If the pressor substance present in the blood of animals with experimental renal hypertension is really hypertensin, it should be destroyed by hypertensinase.

Control experiments were performed first, of which the following are examples.

Twenty units of hog renin were injected intravenously into a normal dog, No. 9-43, and, after 2 minutes, 200 cc. of blood was withdrawn from the jugular vein. The blood was chilled immediately to 0°C. and centrifuged for 1 hour in the cold. The plasma was then separated and left standing at 0°C., for 24 hours. The 120 cc. of plasma was then divided

into two equal parts. The first part was immediately coagulated by heat, after adjustment of pH to 4.5. The second part was first subjected to the action of the hypertensinase in the blood by incubation at 40°C. for 24 hours and then also coagulated by heat, after adjustment of pH to 4.5. Both samples were filtered through Büchner filters, and the pH of the filtrates was adjusted to 7.2. Then the solutions were injected into normal dogs to test for the hyper-

TABLE VI

Hypertensin in Blood of Dogs with Experimental Malignant Renal Hypertension (Both Renal Arteries Greatly Constricted)

Dog No.	Weight	Period of hypertension	Blood pressure	Amount of blood withdrawn	Incubation	Total yield of deproteinized plasma	Amount injected to test for pressor effect	Rise in blood pressure (direct mean femoral)
	lbs.	*days*	*mm.*	*cc.*	*hrs.*	*cc.*	*cc.*	*mm.*
10-39	30	3	220	500	48	200	20	25
9-98	31	2	175	850	48	340	70	30
10-46	29	3	185	70	—	30	30	15
10-30*	21	3	185	190	—	80	80	0
10-45	23	2	205	300	—	120	60	40
							60	50
10-34	23.5	2	175	290	24	120	20	30

* Dog 10-30 was the only one that was not uremic when the sample of blood was taken, but that azotemia alone is not accompanied by the accumulation of renin or hypertensin in the blood is shown by the absence of a pressor substance in the blood of two bilaterally nephrectomized dogs with pronounced azotemia (see below).

Hypertension in Blood of Bilaterally Nephrectomized Azotemic Dogs

Dog. No.	Weight	Period after bilateral nephrectomy	Blood pressure	Amount of blood	Incubation	Total yield	Amount injected to test for pressor effect	Rise in blood pressure (direct mean femoral)
	lbs.	*days*	*mm.*	*cc.*	*hrs.*	*cc.*	*cc.*	*mm.*
10-19‡	44	3	125	200	24	90	90	0
10-56‡	49	2	125	300	24	130	130	0

‡ Both dogs had azotemia at the time the sample of blood was withdrawn for detection of a pressor substance. In dog 10-19 the blood urea nitrogen was 192 mg., the creatinine was 9.5 mg., and the CO_2-combining power was 40.1 volumes per 100 cc., while in dog 10-56 the values were blood urea nitrogen 99.8 mg., creatinine 7.2 mg., and CO_2 43.5 volumes per 100 cc.

tensin present. Part 1 (without hypertensinase action) gave a rise of 60 mm. Hg. Part 2 (with hypertensinase action) gave a rise of 20 mm. Hg.

The same experiment was repeated. About 2 minutes after the intravenous injection of 10 units of hog renin into dog 9-98, 200 cc. of blood was withdrawn. Part 1 (without hypertensinase action) gave a rise of 35 mm. Hg, Part 2 (with hypertensinase action) gave no rise.

In another experiment, the plasma from 200 cc. of blood of a dog with malignant hypertension was treated in the manner described above for the demonstration of hypertensin. Of this plasma, 20 cc. gave a rise of 30 mm. Hg when injected intravenously into a normal

dog. The remaining 60 cc. was divided into three parts. One part was incubated at 40°C. with 10 cc. of normal dog plasma, containing hypertensinase. The hypertensin was completely inactivated in 1 hour. A second part was incubated at 40°C., with a plant hypertensinase preparation, (150 units), derived from wheat bran (8), which does not destroy adrenalin or hydroxytyramine, but does inactivate hypertensin, produced *in vitro*. The hypertensin was completely inactivated in 1 hour. The third part was used as control (not treated) and gave a rise of 30 mm. Hg.

These three tests show that the pressor substance which forms in the systemic blood after the intravenous injection of renin, or after the constriction of the main renal arteries, is destroyed by hypertensinase. Since incubation with plasma containing hypertensinase, or with plant hypertensinase, destroys only hypertensin, and does not affect adrenalin or hydroxytyramine, the pressor substance in the systemic blood of dogs with experimental renal hypertension must be hypertensin or some similar substance.

Chemical Properties of the Pressor Substance in the Systemic Blood of Animals with Experimental Renal Hypertension of the Malignant Type.—Plasma from the systemic blood of dogs with malignant hypertension was used to test for some of the chemical properties which characterize known hypertensin. During the processing of the plasma the substance was subjected to boiling for 10 minutes at pH 4.5, and remained active. Therefore, the substance is heat-stabile and acid-fast. An amount of processed plasma capable of giving a rise of 30 mm. Hg if injected intravenously into an unanesthetized normal dog was boiled for 10 minutes at pH 12.5. After the solution had been cooled, the pH was adjusted to 7.4 with normal HCl. This procedure resulted in complete destruction of the pressor activity. The same amount of processed plasma was dialyzed against cold running tap water for 18 hours. The pressor substance disappeared from the solution in the tubing. The same amount of processed plasma was extracted three successive times with ether, in a separatory funnel, but the pressor activity was not affected by this procedure.

Thus the pressor substance in the circulating systemic blood of dogs with the malignant type of experimental renal hypertension is a non-protein substance, heat-stabile, dialyzable, acid-fast, alkali-labile and ether-insoluble. These qualities are characteristic of hypertensin.

DISCUSSION

Many attempts have been made to demonstrate the existence of a pressor substance in the systemic blood of hypertensive animals:—

Collins and Hoffbauer (9) transfused blood equal to 20 per cent of body weight from a hypertensive dog to a normal dog without obtaining an elevation of blood pressure in the recipient dog.

Katz, Friedman, Rodbard, and Weinstein (10) transfused up to 2500 cc. of blood from a hypertensive dog into a normal dog, by cross-transfusion, without getting a

positive result. Similar experiments, with negative results, have been performed by Houssay and Fasciolo (11). Solandt, Nassim, and Cowan (12) transfused up to 3 liters of blood from hypertensive animals into normal dogs without producing a rise in blood pressure, but they did observe an elevation of blood pressure when such large amounts of blood were transfused into bilaterally nephrectomized animals.

Although I. H. Page (13) was unable to show any pressor effect in 60 cc. of blood from hypertensive dogs, yet later he was able to detect a vasoconstrictor substance in 0.4 cc. of plasma from dogs with experimental renal hypertension, if it was perfused through an isolated rabbit's ear. There is no proof that this effect was due to hypertensin and the work has not been confirmed by others (14).

Heymans and Bouckaert could not demonstrate a pressor substance in up to 20 cc. of blood from hypertensive dogs (15), and others (18, 19) have failed to find hypertensin in the blood of hypertensive patients or animals, or in the blood of animals after an intravenous injection of renin.

The failure of all these attempts to detect the pressor substance in the systemic blood of dogs with experimental renal hypertension can be explained by the inadequate amounts of blood tested and the failure to avoid destruction of the pressor substance by the action of hypertensinase during the tests. Dell'Oro and Braun-Menendez (16) detected renin in both the renal venous blood and in blood from the femoral artery of dogs with experimental renal hypertension that had lasted only a few days. They detected it in as little as 12 cc. of plasma by adding hypertensinogen to enhance the action of the renin and by testing for the hypertensin formed. On this account, their estimate about the amount of renin (100 to 200 units) constantly circulating in the systemic blood of a dog in the earliest stage of experimental renal hypertension is probably too high. The results of our study show that pressor substance is present in small quantity in the circulating blood; therefore, even in the earliest stage, but especially in the later stage of the benign phase of experimental renal hypertension, a large amount of blood must be tested if the hypertensin is to be detected without enhancing the action of renin by the addition of hypertensinogen. This amount corresponds to from one-fifth to one-third of the animal's blood. But even then the pressor substance cannot be demonstrated if the action of the hypertensinase of the plasma is not inhibited. Since 1 cc. of plasma contains almost 1 unit of hypertensinase (17), the 300 to 400 units of hypertensinase can quickly destroy the 1 unit of hypertensin present in this amount of blood, if no precautions are taken to prevent this reaction. If the blood is even slightly hemolyzed, much greater amounts of hypertensinase are available for the destruction of the small amount of hypertensin present in the circulating systemic blood, because red blood corpuscles are rich in hypertensinase, and the detection of hypertensin is therefore likely to fail.

SUMMARY

1. A method has been developed which makes possible the demonstration of a pressor substance in the circulating systemic blood of dogs with experimental renal hypertension.

2. After the intravenous injection of renin into normal dogs, it was possible to detect a pressor substance formed in the systemic blood. After the intravenous injection of 1 unit of renin, as much as 1 unit of the pressor substance was detected in the plasma from 200 cc. of systemic blood.

3. Large amounts of systemic blood pooled from several normal dogs did not contain detectable amounts of pressor substance.

4. In experimental renal hypertension due to unilateral or bilateral constriction of the main renal arteries, a pressor substance was demonstrated in large amounts of systemic blood, corresponding to from one-fifth to one-third of the total blood volume. This was accomplished without the addition of hypertensinogen to enhance the action of the renin in the blood. In an animal weighing about 15 kilos, with benign hypertension up to 3 months' duration, about 3 to 5 units of this pressor substance are probably constantly circulating in the entire systemic blood.

5. The pressor substance was also detected in a relatively small amount of renal vein blood from an ischemic kidney.

6. In the systemic blood of dogs weighing about 15 kilos, with malignant experimental renal hypertension, from 15 to 25 units, or more, of the pressor substance are present in the entire circulating blood.

7. The pressor substance which appears in the systemic blood of dogs with experimental renal hypertension, and of normal dogs after intravenous injection of renin, is destroyed by hypertensinase.

8. The pressor substance obtained from the systemic blood of dogs with experimental renal hypertension has the same physiological and chemical properties as hypertensin produced *in vitro*. It is therefore suggested that the name *hypertensin* be adopted for the pressor substance which causes experimental renal hypertension.

9. In this study the animals in the benign phase of hypertension were almost all in the early stage (3 months or less). Whether the humoral mechanism obtains in animals in the late stage, after years of hypertension, or in any form of human hypertension is being investigated.

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